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Effect of Ibogaine on Naloxone-Precipitated Withdrawal Syndrome in Chronic Morphine-Dependent Rats

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Abstract—Ibogaine, an indole alkaloid, administered intracerebroventricularly 4–16 μ g, attenuated a naloxone-precipitated withdrawal syndrome in chronic morphine-dependent rats. It appears that ibogaine has a more consistent effect on certain selective withdrawal signs related to the locomotion. This might explain an attenuating effect of ibogaine on some withdrawal signs. However, due to complex interaction of ibogaine with serotonin and other neurotransmitter systems, the mechanism of ibogaine antiwithdrawal effect remains unknown and requires further elucidation.

Introduction

There is some evidence that brain serotonin (5-HT) can modulate an opiate withdrawal syndrome. Way *et al.* (1968) and Ho *et al.* (1973) demonstrated that inhibition of 5-HT synthesis or destruction of 5-HT system might significantly attenuate a naloxone-precipitated withdrawal syndrome. Because some of these results were not confirmed (Cheney and Goldstein, 1971; Johnson *et al.*, 1978), the relationship between the brain serotonin system and the opiate withdrawal syndrome has become controversial. Interest in this problem had increased again since recent data have shown a decrease or increase of severity of morphine withdrawal in animals after pretreatment with 5-HT₂ antagonists (Cervo *et al.*, 1981; Neal and Sparber, 1986) or with 5-HT releaser (Samanin *et al.*, 1980), respectively.

Ibogaine is an indole alkaloid of the family apocynaceae, with central

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stimulant (Schneider and Sigurdson, 1956) like properties (Schneider and Sigurdson, 1956) blocking drug metabolism (Schneider and Sigurdson, 1956) between 5-HT synthesis and release (Schneider and Sigurdson, 1956) animals might be of interest (Schneider and Sigurdson, 1956) ibogaine potentiated the withdrawal syndrome (Schneider and Sigurdson, 1956) and McArthur, 1978) interfering with many of the effects of morphine (Schneider and Sigurdson, 1956) ibogaine is a strong anticonvulsant (Schneider and Sigurdson, 1956) 1956) and its action on the central nervous system (Schneider and Sigurdson, 1956) potentiated the precipitation of morphine withdrawal (Schneider and Sigurdson, 1956) Ibogaine has been used in the treatment of morphine addiction (Schneider and Sigurdson, 1956) 1968; Sloviter *et al.*, 1978) suggested that ibogaine might have a psychological aspect to its action (Schneider and Sigurdson, 1956) these tentative conclusions are based on the naloxone-precipitated withdrawal syndrome in morphine-dependent rats. The mechanism of the antiwithdrawal effect of ibogaine in these trials of ibogaine in morphine-dependent rats requires further clarification.

Methods

Adult male Wistar-Kyoto rats (Wistar-Kyoto, Taconic, Møllegaard, Denmark) weighing 200–250 g were used. Food and water were available *ad libitum*. The rats were administered intracerebroventricularly (i.c.v.) a solution of ibogaine (0.15 ml/100 g, s.c.), implanted with stainless steel cannulae (26 gauge, 0.15 ml/100 g, s.c.), described in an earlier publication (Schneider and Sigurdson, 1956) nula was carried out in a stereotaxic apparatus (Kopf, Tübingen, Germany) using a stainless steel cannula (26 gauge, 0.15 ml/100 g, s.c.), lateral ventricle (Kopf, Tübingen, Germany) into the ventricle using a stainless steel cannula (26 gauge, 0.15 ml/100 g, s.c.) microsyringe by the method of Wajsborn (1978) the lateral ventricle. In each experiment, up to 10 rats were used. The cannulae were implanted by Paakkari (1980) and the injection needle was inserted into the i.c.v. cannula during the experiment. The animal on the stereotaxic apparatus was fixed in a stereotaxic apparatus.

stimulant (Schneider and Sigg, 1957; Singbartl *et al.*, 1973) and serotonin-like properties (Sloviter *et al.*, 1980), which can be antagonized by 5-HT blocking drug methysergide (Dhahir, 1971). Therefore, an interaction between 5-HT system and opiate withdrawal in ibogaine pretreated animals might be of importance. In addition, it has been demonstrated that ibogaine potentiated morphine analgesia in mice and humans (Schneider and McArthur, 1956). Ibogaine, however, exerts a complex activity interfering with many other neurotransmitter systems. It has been reported that ibogaine is a strong inhibitor of serum cholinesterase (Hamet and Rothlin, 1956) and its arousal-inducing effects can be blocked by atropine (Schneider and Sigg, 1957). Gershon and Lang (1962) found that ibogaine potentiated the pressor response to both adrenaline and noradrenaline. Ibogaine has been described as a hallucinogenic substance (Farnsworth, 1968; Sloviter *et al.*, 1980). Unpublished results by H. Lotsof (New York) suggested that ibogaine given per os may interrupt the physiological and psychological aspects of the opiate withdrawal syndrome in humans. Given these tentative conclusions, it seems useful to examine the effect of ibogaine on the naloxone-precipitated withdrawal syndrome in chronic morphine-dependent rats. These experiments elucidate the effect of ibogaine on the opiate withdrawal syndrome and might provide some basis for clinical trials of ibogaine for the treatment of opiate dependence. However, the mechanism of the antiwithdrawal action of ibogaine remains unknown and requires further clarification.

Methods

Adult male Wistar rats (190–200 g) were used. The animals were housed singly. Food and water were available *ad libitum*. Ibogaine was administered intracerebroventricularly (i.c.v.). All animals were chronically implanted with stainless steel cannula in the left lateral cerebral ventricle as described in an earlier paper (Dzolic *et al.*, 1979). An implantation of cannula was carried out under hypnorm anaesthesia (fluanison/fentanyl base, 0.15 ml/100 g, s.c.). The i.c.v. administration of drugs was performed by using a stainless steel cannula stereotactically directed 1 mm in the left lateral ventricle (König and Klippel, 1963). Drug solutions were injected into the ventricle with needle gauge 30, attached to a Hamilton microsyringe by polyethylene tube. The needle was protruded 1 mm into the lateral ventricle. Correct ventricular cannulation was verified before each experiment, using a modification of the technique previously described by Paakkari (1980). In this procedure a polyethylene tube was attached to the injection needle and filled with saline. To test the correct placement of i.c.v. cannula during surgery, the tube was raised above the head of the animal on the stereotaxic apparatus, and a rapid inflow of saline denoted a

correct placement of cannula. At least 6 days recovery were allowed before the experiments. Each rat was tested only once.

For inducing chronic opiate dependence, morphine pellets (85 mg) were implanted subcutaneously (s.c.) on the back of the animals under ether anaesthesia (Blasig *et al.*, 1973). The opiate withdrawal syndrome was precipitated 72 hr after the pellets implantation by naloxone (5 mg/kg, i.p.) dissolved in saline. The withdrawal syndrome was induced only once in each rat. The behavior of the rat was observed in a plastic box (base area: 25 × 40 cm, height: 15 cm). Ibogaine, in a dose range 4–16 µg, was administered i.c.v. 15 min prior to naloxone. Doses referred to the salts and pH was kept about 6.6–7.0. The control group received the same volume of artificial cerebrospinal fluid (CSF). The composition of the CSF/l is as follows: NaCl 8.10 g; KCl 0.25 g; CaCl₂ 0.14 g; MgCl₂ 0.11 g; NaHCO₃ 0.18 g; NaH₂PO₄ 0.07 g; urea 0.13 g; glucose 0.61 g. The observation period started at the time of i.c.v. injection of CSF or ibogaine and lasted up to 30 min after naloxone administration. Withdrawal signs were counted and checked. Jumping consisted of animals leaping on the metal rack, which covered the box, with all 4 feet off the ground. Other withdrawal signs have clear meaning.

Drugs used were naloxone-hydrochloride (SIGMA), morphine-sulphate (Diosynth) and ibogaine-hydrochloride (kindly donated by H. Lotsof, NDA, New York). Ibogaine and naloxone were dissolved in CSF or saline, respectively.

Statistical analysis was conducted to explore the difference in the 3 experimental groups and the control group over 21 distinct withdrawal signs. To ascertain significant difference a nonparametric analysis of variance (Kruskal-Wallis) was performed. The significance of the difference between the significantly different group means were evaluated by Mann-Whitney U-test (Saxena, 1985). Significance was accepted at $p < 0.05$.

Results

Effect of ibogaine on morphine-dependent animals

A tremor observed in mice by Singbartl *et al.* (1973) was not found in the ibogaine-treated rats within a dose range of 4–16 µg. The behavioral signs of addicted naive rats common with the opiate withdrawal syndrome (but in low frequency) have not been significantly altered by i.c.v. administration ibogaine. Thus, no marked changes were found in behavioral signs such as: digging, head shakes, scratching, grooming, drinking, eating, penile licking and ejaculation. However, rearing was significantly decreased (not shown).

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Effect of ibogaine on morphine withdrawal syndrome

Administration of naloxone (5 mg/kg, i.p.) 15 min after CSF (i.c.v.) in chronic morphine-dependent rats induced a withdrawal syndrome (Table I). The i.c.v. administration of ibogaine (4–16 μ g), 15 min prior to naloxone (5 mg/kg, i.p.), significantly reduced, in a dose-related manner, the frequency of the following symptoms: rearing, digging, head hiding, chewing, teeth chattering, writhing, jumping and salivation. The frequency of other withdrawal signs were nonsignificantly decreased or not altered: "wet-dog" shakes, head shakes, stretching, grooming, scratching,

TABLE I

The effects (mean $\pm$ S.E.M. or response ratios ⁽¹⁾) of i.c.v. injection of increasing doses of ibogaine (4, 8 and 16 μ g/rat) on withdrawal syndrome precipitated by naloxone (5 mg/kg, i.p.) in chronic morphine-dependent rats

Withdrawal sign	CSF (n = 10)	Ibogaine (μ g/rat, i.c.v.)			
		4 (n = 10)	8 (n = 10)	16 (n = 10)	
Rearing	20.30 $\pm$ 1.28	15.70 $\pm$ 1.86*	14.50 $\pm$ 1.17*	14.70 $\pm$ 1.43*	
Digging	10.20 $\pm$ 2.67	5.20 $\pm$ 1.05*	4.20 $\pm$ 0.76*	4.20 $\pm$ 1.02*	
Head hiding	6.60 $\pm$ 0.70	4.10 $\pm$ 2.14*	2.70 $\pm$ 1.26*	2.00 $\pm$ 0.70*	
"Wet-dog" shakes	11.70 $\pm$ 1.85	10.20 $\pm$ 0.99	9.60 $\pm$ 1.27	9.20 $\pm$ 1.64	
Head shakes	3.10 $\pm$ 0.55	2.70 $\pm$ 0.65	2.60 $\pm$ 0.65	2.60 $\pm$ 0.52	
Chewing	55.50 $\pm$ 5.17	35.10 $\pm$ 5.13*	23.50 $\pm$ 1.81*	31.40 $\pm$ 4.31*	
Teeth chattering	48.60 $\pm$ 7.26	24.50 $\pm$ 6.90*	23.50 $\pm$ 1.81*	23.70 $\pm$ 5.69*	
Writhing	5.60 $\pm$ 1.06	3.20 $\pm$ 0.39*	1.80 $\pm$ 0.39*	1.60 $\pm$ 0.99*	
Stretching	0.70 $\pm$ 0.30	0.60 $\pm$ 0.27	0.60 $\pm$ 0.22	0.70 $\pm$ 0.30	
Grooming	6.70 $\pm$ 1.58	6.00 $\pm$ 0.88	4.60 $\pm$ 1.49	4.80 $\pm$ 0.51	
Scratching	1.20 $\pm$ 0.61	1.10 $\pm$ 0.53	0.90 $\pm$ 0.50	0.40 $\pm$ 0.15	
Penile licking	1.90 $\pm$ 0.46	4.00 $\pm$ 0.85*	4.10 $\pm$ 0.63*	6.80 $\pm$ 1.25*	
(mean $\pm$ S.E.M. – counted signs)					
Jumping	0.7	0.5	0.2*	0.2*	
Vocalization	0.7	0.7	0.5	0.6	
on touch					
Ptosis	0.7	0.6	0.4	0.4	
Diarrhea	0.7	0.6	0.4	0.4	
Urination	0.2	0.2	0.1	0.1	
Rhinorrhea	0.5	0.2	0.1	0.1	
Salivation	0.7	0.5	0.2*	0.2*	
Paw tremor	0.1	—	—	0.1	
Ejaculation	0.3	0.4	0.6	0.6	
(response ratio – checked signs)					

The control group received artificial cerebrospinal fluid (CSF) instead of an ibogaine.

⁽¹⁾ = The number of rats displayed checked sign per number tested.

* = Significant difference compared to control ($p < 0.05$).

vocalization on touch, ptosis, diarrhea, urination, rhinorrhea, paw tremor and ejaculation. However, the frequency of penile licking was significantly increased (Table I). In addition, all animals pretreated with ibogaine demonstrated during withdrawal a decreased locomotion occasionally associated with tremor.

Discussion

Ibogaine decreased the motor activity in addicted animals during a naloxone-precipitated withdrawal syndrome. Since ibogaine is a rather strong central stimulant (Schneider and Sigg, 1957) a lack of increased motor activity following ibogaine is in contrast to observations of many other central stimulants. A reduction of locomotion induced by ibogaine might explain an inhibitory effect of this drug on the rearing, digging and jumping during naloxone-precipitated withdrawal.

In addition, this study shows that i.c.v. injection of ibogaine attenuates several other withdrawal symptoms in chronic-morphine-dependent rats such as: head hiding, chewing, teeth chattering, writhing and salivation. In general, rats had less desire to hide or to escape. A tremor, described in male mice after s.c. administration of ibogaine (Zetler *et al.*, 1972), was not observed after i.c.v. administration of this drug in morphine-dependent rats. However, a tremor was occasionally registered during naloxone-precipitated withdrawal of ibogaine pretreated (i.c.v.) rats.

The mechanism of attenuation of morphine-withdrawal signs in the rats following ibogaine administration is not clear. This indole alkaloid possesses a very complex activity on neurotransmitter systems, affecting both the noradrenergic (Gershon and Lang, 1962) and the cholinergic systems (Hamet and Rothlin, 1956). It has also been found that ibogaine, as hallucinogen, shares the common mechanism of action as lysergic acid (LSD), activating central 5-HT receptors (Sloviter *et al.*, 1980). Accordingly, methysergide, a 5-HT₂ receptor blocking drug, reduced the hypotensive effects of both ibogaine and 5-HT (Dhahir, 1971). These interactions of ibogaine and the 5-HT system might explain the stimulatory effect of this indole alkaloid on sexual phenomena in opiate withdrawal syndrome. In these experiments, ibogaine significantly increased penile licking. This confirms the recent data which have shown that activation of 5-HT₁ receptors facilitates the expression of sexual behaviour in male rats (Mendelson and Gorzalka, 1986). However, the intimate interaction of ibogaine with different 5-HT receptor populations is still unknown. The situation is further complicated by the fact that the intimate mechanism of opiate withdrawal is also unknown. However, this study shows that ibogaine has a consistent effect on certain selective withdrawal signs having to do with

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locomotion. It seems promising to conduct more experimentation on the specific effects of ibogaine on the serotonin system and on selective parts of the opiate withdrawal syndrome.

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